A common European approach to the regulatory testing of nanomaterials

Demonstration of a probe-sonicator calibration protocol for harmonizaton of batch dispersions used for toxicological testing

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SEVENTH FRAMEWORK PROGRAMME

The problem

Testing the test: Can we do it better?

- **Variability is observed in (eco-)toxicological test results**
  - Material variability?
  - Exposure characteristics (agglomerates vs dispersed)?
  - Biological variability?
  - Variability in medium characteristics?
  - Variability in the assay – scales ?
  - Variability in the analytical methods?
  - Differences in preparation methods

Examples of difference due to test item preparation procedure

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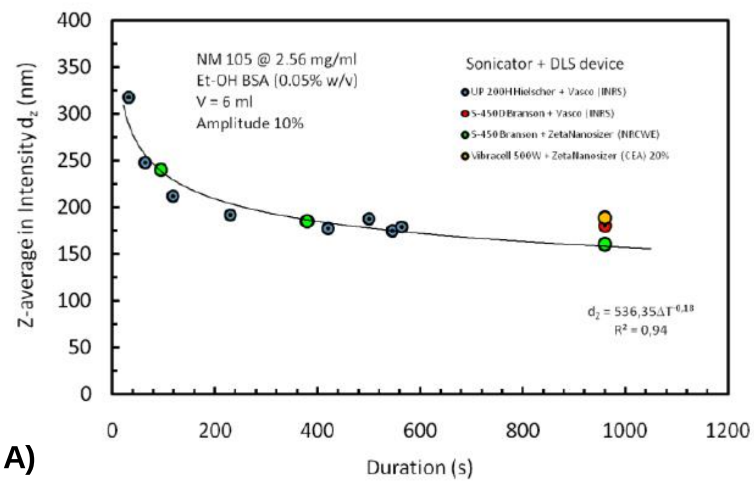
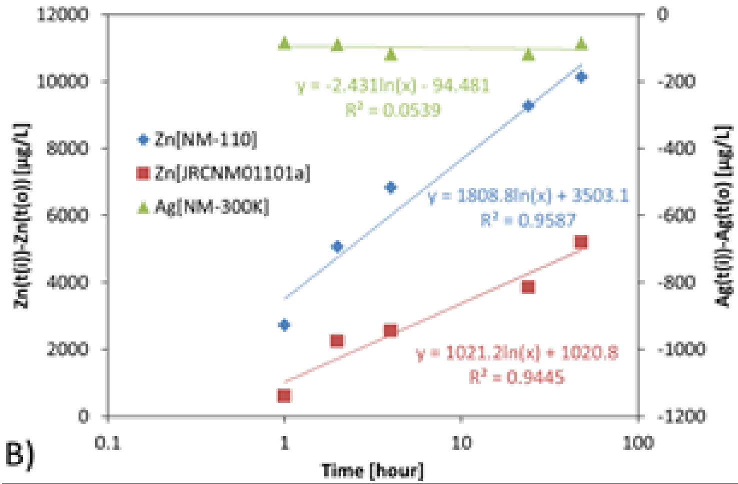


A)



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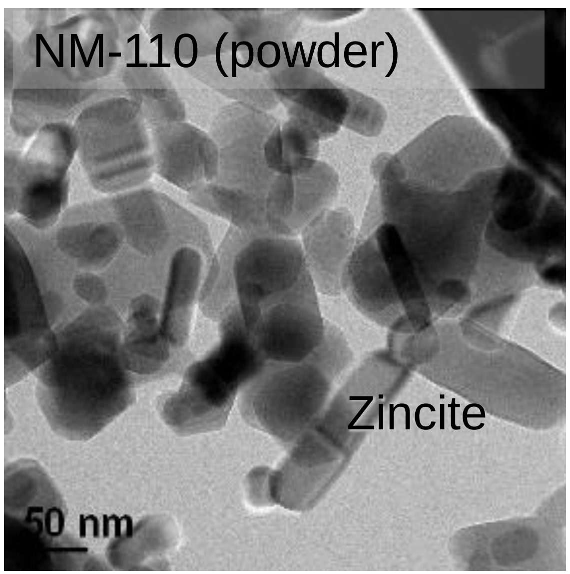
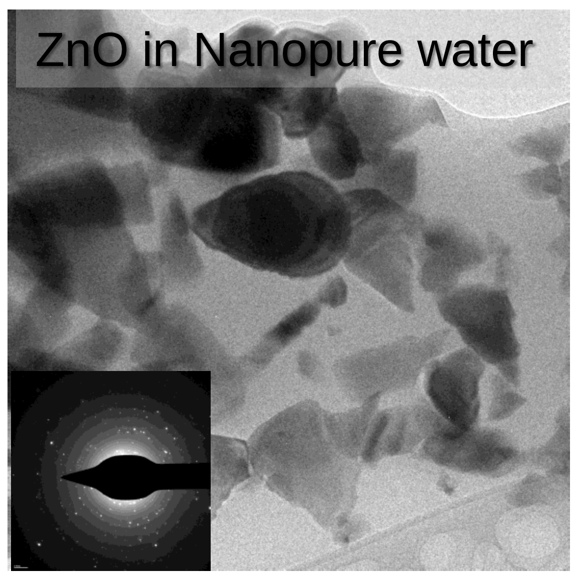
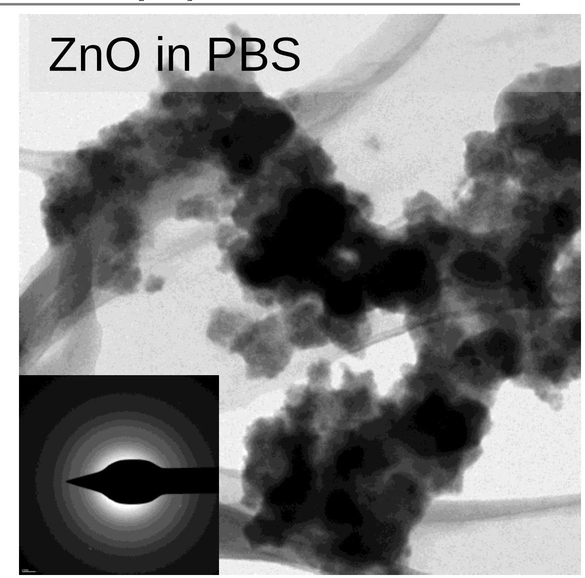




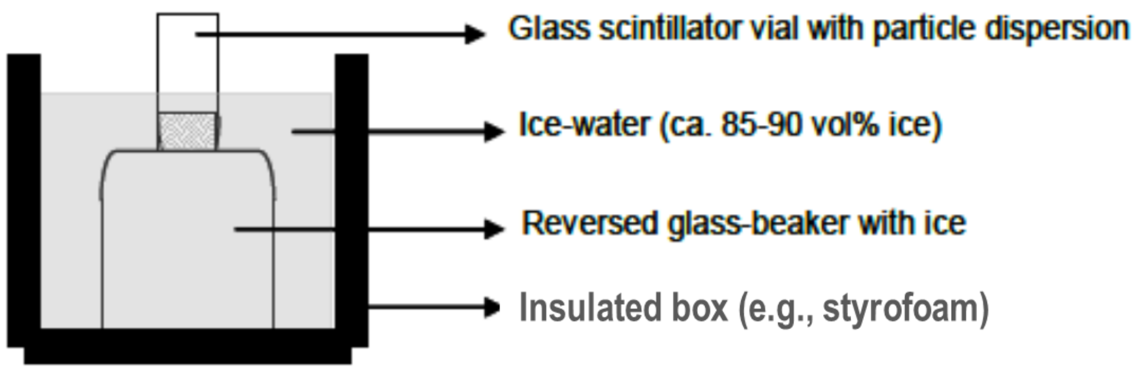


The NANOGENOTOX generic probe-sonicator dispersion protocol

- Pre-wetting in 50 µL Ethanol

How can we reach comparability in exposure and test results in many different lab’s (20+)?

- Characterize dispersion by DLS (and microscopy)



The conceptual approach

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Probe-sonicator calibration protocol

Harmonize de-agglemeration energies/efficiencies

Batch dispersion protocols

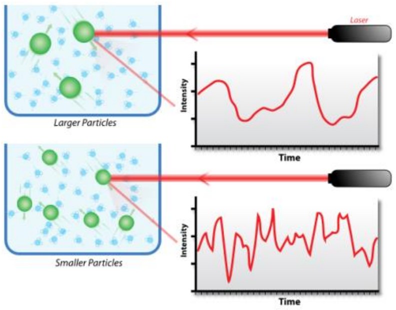
Harmonize Initial Exposure Characteristics (per protocol)

In vivo

In vitro

ecotoxicology

DLS



Exposure characterization methods and protocols

Harmonized reporting to enable comparative analysis

Interpretation, interpolation, extrapolation, read-across ….

Calibration of probe-sonicators

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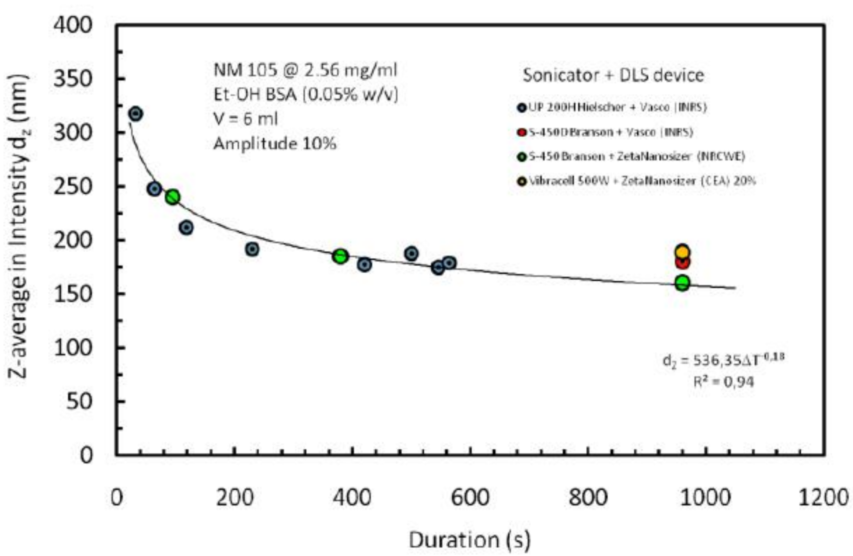
Requirements for implementation
Easy-to-use and not very time-consuming
Reliable in the sense of giving a trustworthy result and being repeatable
Apply tools that are available in "all" nanotoxicology laboratories

Performance-calibration using benchmark material



Calorimetric calibration of acoustic delivered energy

Jensen et al. Final protocol for producing suitable MN exposure media. (June 2011). www.nanogenotox.eu
Taurozzi et al. Preparation of nanoparticle dispersions from powdered material using ultrasonic disruption. *National Institute of Standards and Technology*. 2012.

The calibration protocol

1) Identifying the amplitude/duration to reach the acoustic delivered target energy

$$7.35 \pm 0.05 = P_{ac}\left(Watt\right) = \frac{\Delta T}{\Delta t} MC_p \mapsto \left(16\,\mathrm{min}\right)$$

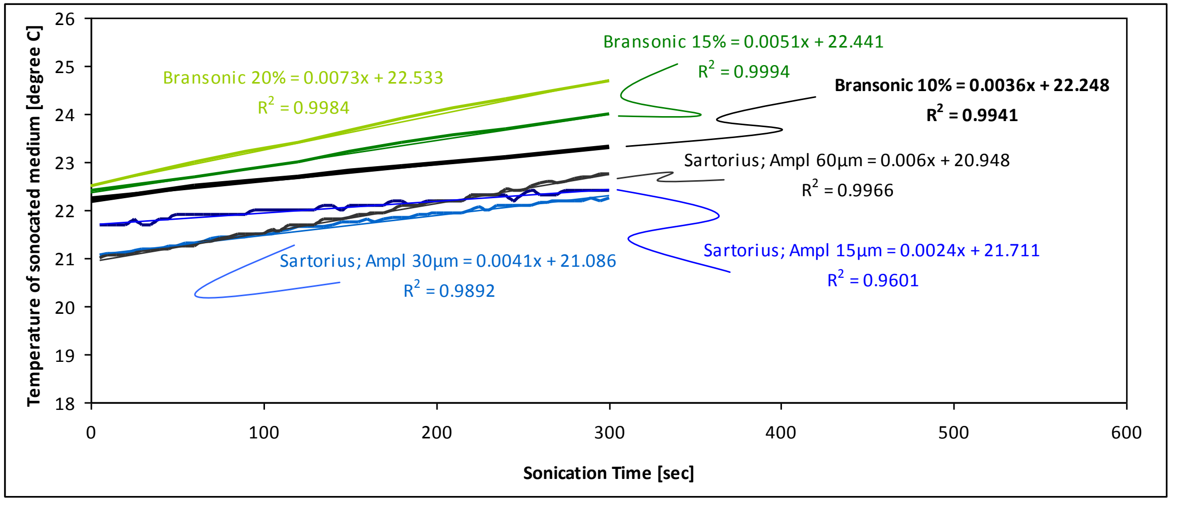
2) Verification of dispersion state using the DLS Zeta-average size of NM-200 (SAS) as benchmark material (NANOGENOTOX SOP)

$Z_{\mathrm{ave,mean}}$ = 210 to 270 nm and $\mathrm{PDI}_{\mathrm{mean}} < 0.46$ (n = 10 x 3)

3) Adjustment (if needed) of amplitude and/or duration to reach the target range of NM-200

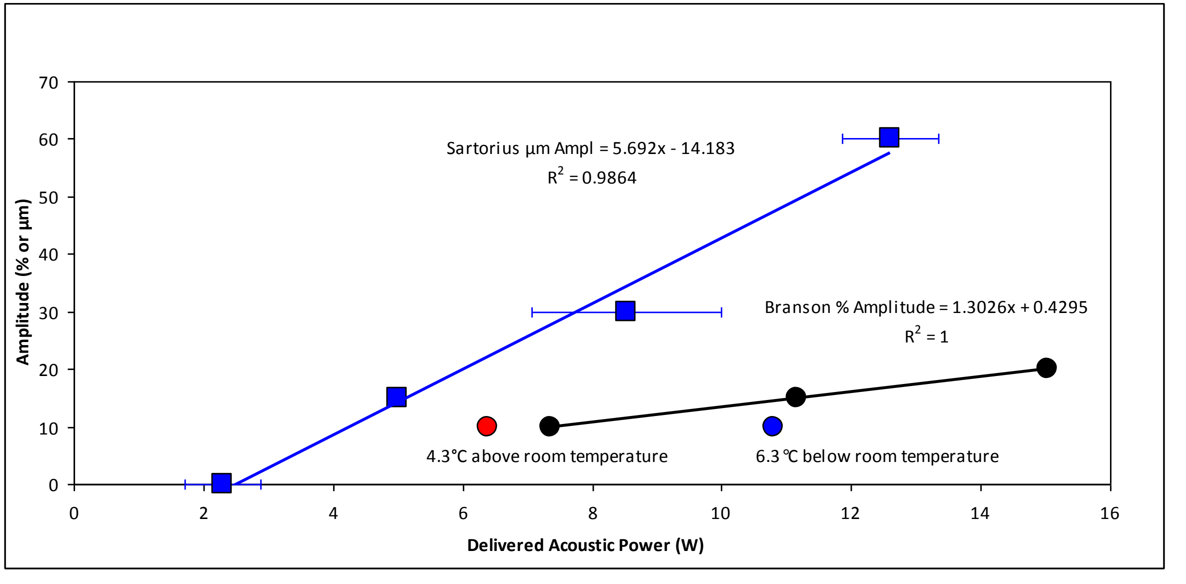
Determination of the acoustic delivered energy

$$\frac{\Delta T}{\Delta t}(n \geq 3)$$



0.5 L MilliQ-filtered water
600 mL beaker
Thermometer ± 0.1°C
Scale to determine (M)

$$P_{ac}(Watt) = \frac{\Delta T}{\Delta t} MC_p$$



Use the regression curve for Amplitude vs. P_{ac} to determine the amplitude setting to reach 7.35Watt

Adjust time of sonication if P_{ac} cannot be reached

Reaching the DLS performance criteria

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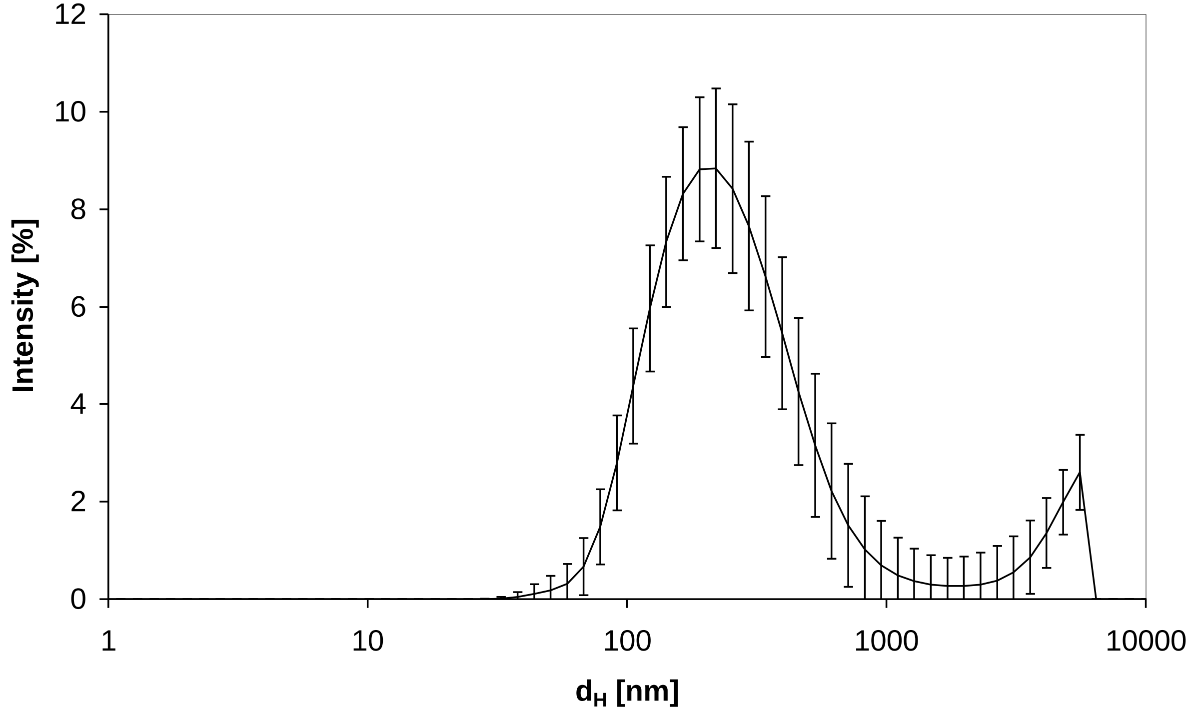
Use identified sonicator amplitude and duration to prepare NM-200 batch dispersions according to the NANOGENOTOX dispersion protocol (n=3)

Measure the hydrodynamic diameter of the NM-200 batch dispersion (n=10 x 3)

If not within range (210 – 270 nm; PDI ≤ 0.46), adjust duration (or amplitude) to reach target values

Repeat preparation of batch dispersions

The "mother data" from NRCWE



n=100	Z(ave)	PDI
NM200	238	0.40
sigma	14	0.06

Interlaboratory performance testing

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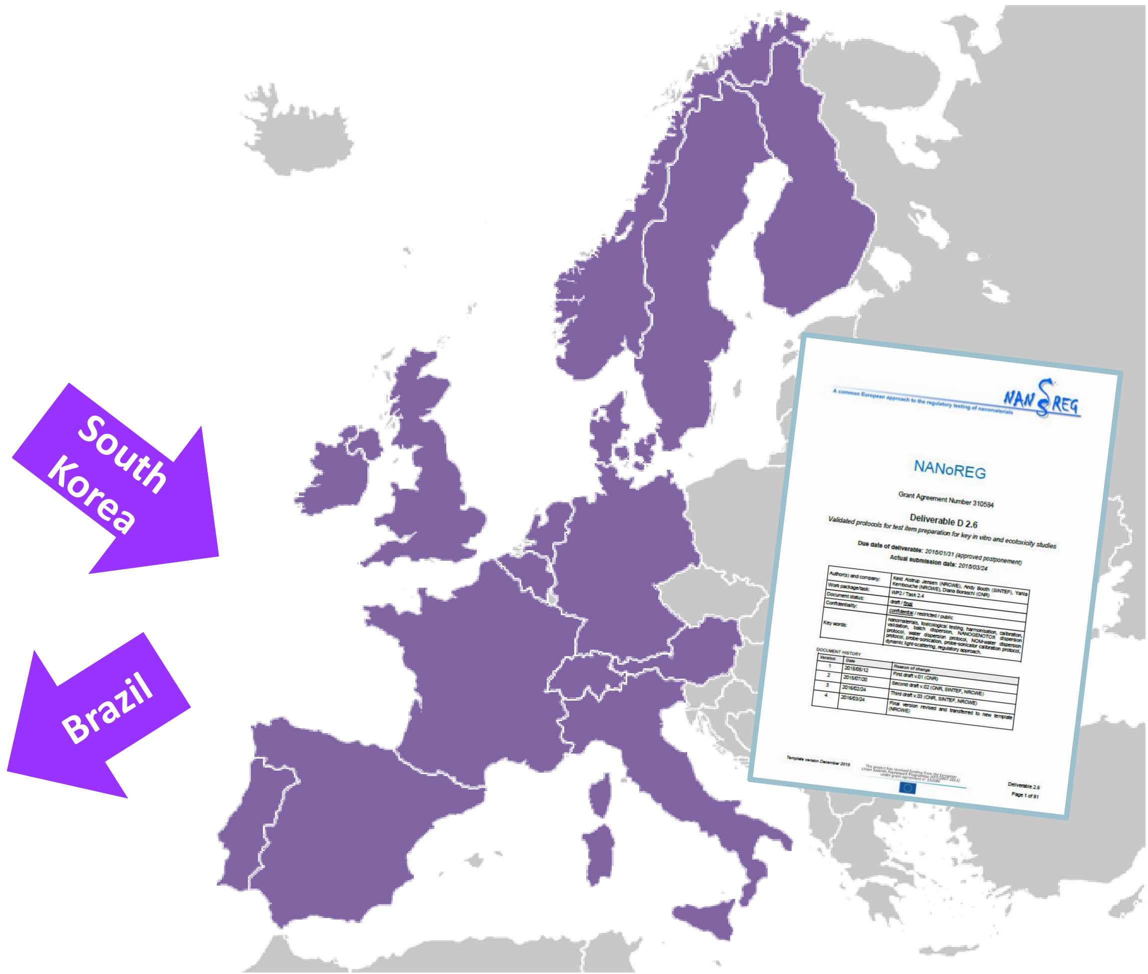
NRCWE
SINTEF
CNR
UdL
LEITAT
IK4-Tekniker
NMBU
IIT
INAIL
INERIS
NPL
STAMI
UIB
INSA
ISS
INMETRO
KI
WUR
UNITO
KRISS
DWE Korea
UAB
ANSES
UNamur
CEA
UIB
UNITO
DTU-Food* (NANODEFINE)



Many different sonicators

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Partner number	Probe sonicator	Watt	KHz	probe diameter
1a	400 Watt Branson Sonifier S-450D	400	20	13
1b	400 Watt Branson Sonifier S-450D	400	20	13
2	400 Watt Branson Sonifier S-450D	400	20	13
3	Bandelin, SONOPULS HD 2200	200	20	9
4	Heat Systems Sonicator Ultrasonic Processor XL (Pulsar or Continuous)	100	20	6
5b P1	Microson XL 2000, Qsonica, LLC	100	22.5	3.2
5c P2	Microson XL 2000, Qsonica, LLC	100	22.5	4.8
5a P4	Microson XL 2000, Qsonica, LLC	100	22.5	6.4
6	Sonics VC750	750	20	13
7	Heat System Misomix XL2020	550	20	13
8	QSONICA Q700 (with probe)	100	20	12.7
9	400 Watt Branson Sonifier S-450D	400	20	13
10	Vibracell ultrasonifier Sonics and Materials Inc, USA	750	20	13
11	MSE Soniprep 150 (UK)	150	50	9.5
12	SONICS Vibra Cell VCX750	750	20	13
13	Misonix Sonicator 3000	400	20	13
14	400 Watt Branson Sonifier S-450D	400	20	13
15	SONICS Vibra Cell VCX750	750	20	13
16	BransonS-450D	200	10	13
17	Branson 250S	200	20	13
18	VibraCell VXC130	130	20	6
19	SCIENTZ-IID	950	20	NA
20	Qsonica - Q700	700	20	3.2
21	Bandelin Sonopuls HD 3100	400	20	3
22	BransonS-450D	400	20	13
23	Branson SLPe	150	20	6.4
24	Qsonica - Q700	700	20	13
25	Vibra-Cell (Model VC 505,Sonics and materials,Ct,USA)	500	20	13
26	UP 400S Sonicator (Hielscher)	400	24	7
27	Branson Sonifier S-450D	400	20	13

Examples of acoustic energy curves

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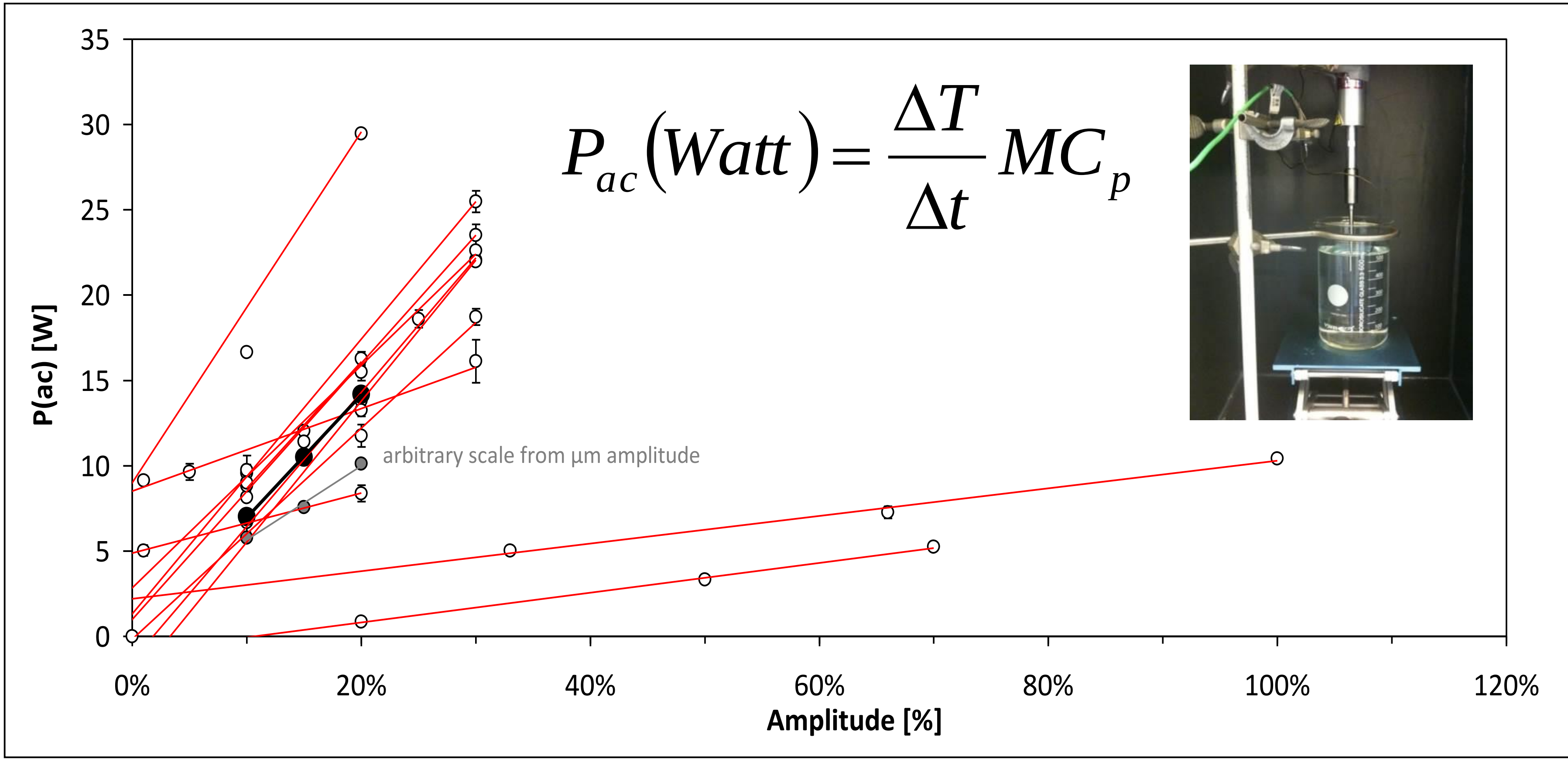
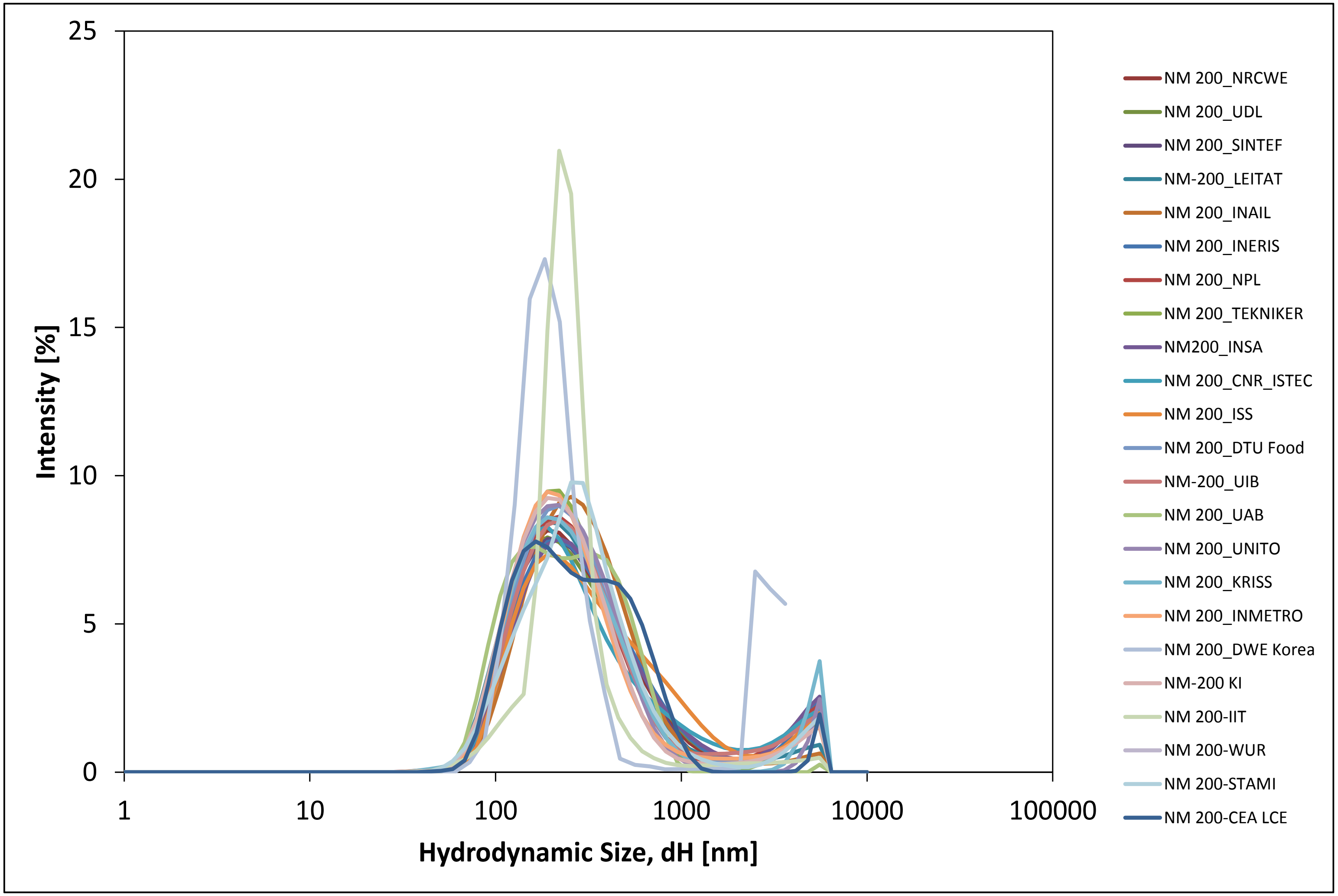


The DLS hydrodynamic size-distributions at calibrated settings



Calibration possible!

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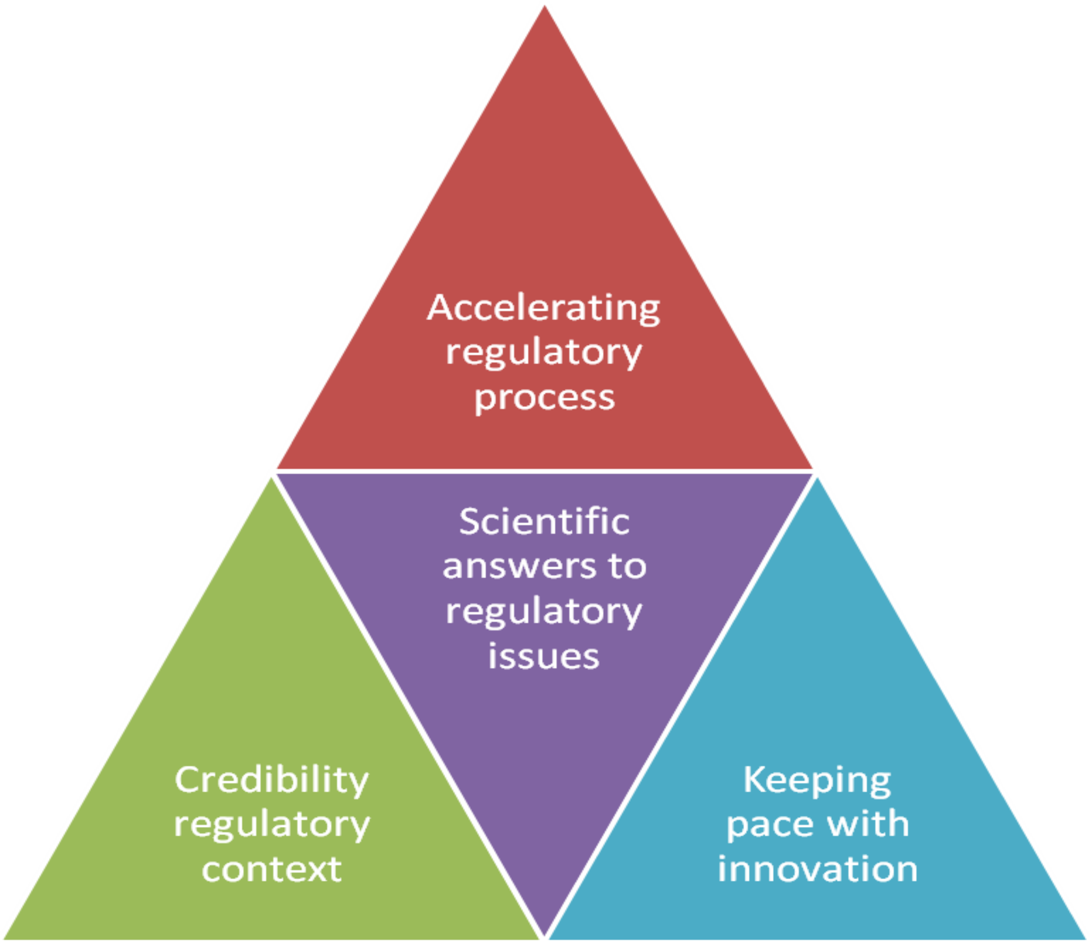
Partner	Z_{ave}	σ	PDI	σ	n
1	250.6	19.7	0.419	0.070	11x10
2	218.1	2.7	0.329	0.029	3x10
3	254.0	13.9	0.402	0.042	3x10
4	250.2	10.3	0.413	0.040	3x10
5	251.1	16.5	0.418	0.058	6x10
6	264.9	41.1	0.337	0.095	3x10
7	248.9	16.8	0.282	[illegible]	[illegible]
8	266.6	18.4	0.414	[illegible]	[illegible]
9	243.5	10.4	0.384	[illegible]	[illegible]
10	264.7	31.1	[illegible]	[illegible]	3x10
11	214.0	5.3	[illegible]	[illegible]	3x1
12	246.4	18.0	[illegible]	[illegible]	3x10
13	#271.8	15.7	[illegible]	0.049	3x10
14	*280.1	[illegible]	[illegible]	0.027	3x10
15	231.4	[illegible]	[illegible]	0.028	3x10
16	222.0	[illegible]	[illegible]	0.039	3x10
17	[illegible]	[illegible]	0.341	0.019	2x10
18	[illegible]	[illegible]	0.371	0.027	3x10
19	[illegible]	[illegible]	0.459	0.042	3x10
20	[illegible]	6.1	0.505	0.024	3x10
21	[illegible]	3.1	0.167	0.013	3x10
22	#272.4	11.4	0.380	0.020	2x3
23	#272.3	10.0	0.430	0.042	1x10
24	CPS disc centrifuge				
25	*303.6	17.4	0.526	0.043	3x10
Average	**252.7**	**13.7**	**0.384**	**0.038**	
σ	**24.9**		**0.079**		

Mission completed


Accelerating regulatory process
Scientific answers to regulatory issues
Credibility regulatory context
Keeping pace with innovation

?

Performance testing and benchmarking

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Type of MNM	MNM Identification codes used by NANoREG*
Titanium Dioxide	NM-100, NM-101, NM-103
Silicon Dioxide	NM-200, NM-203
Zinc Oxide	NM-110, NM-111
Cerium Dioxide	NM-212
Barium Sulphate	NM-220
Silver	NM-300K, NM-302
Nanotubes (single and multi-walled)	NM-400, NM-401, NM-411
Nanofibrillar cellulose	NFC Fine, NFC Medium-coarse, UPM Biofibrils AS, UPM Biofibrils NS, UPM Bleached Birch Pulp

Performance on granular MNM

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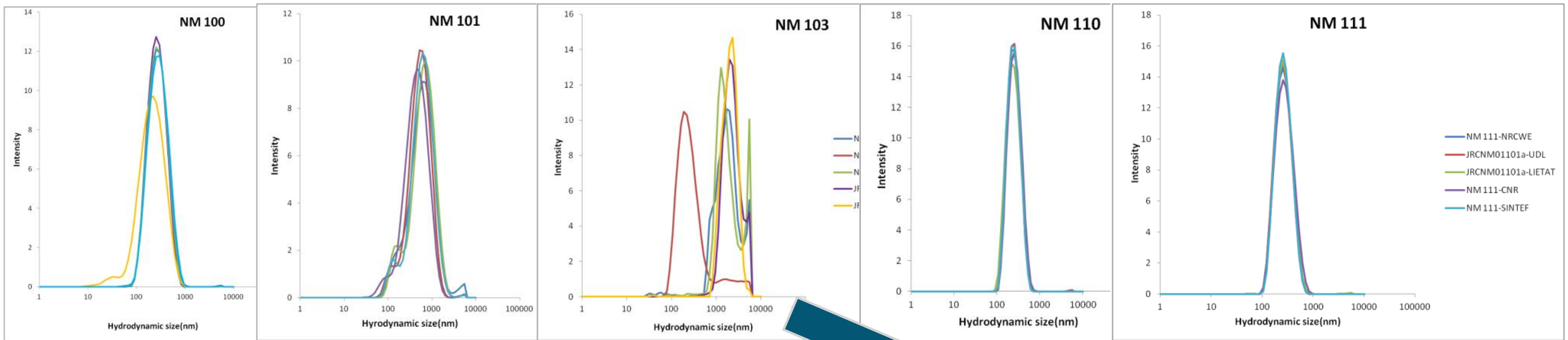

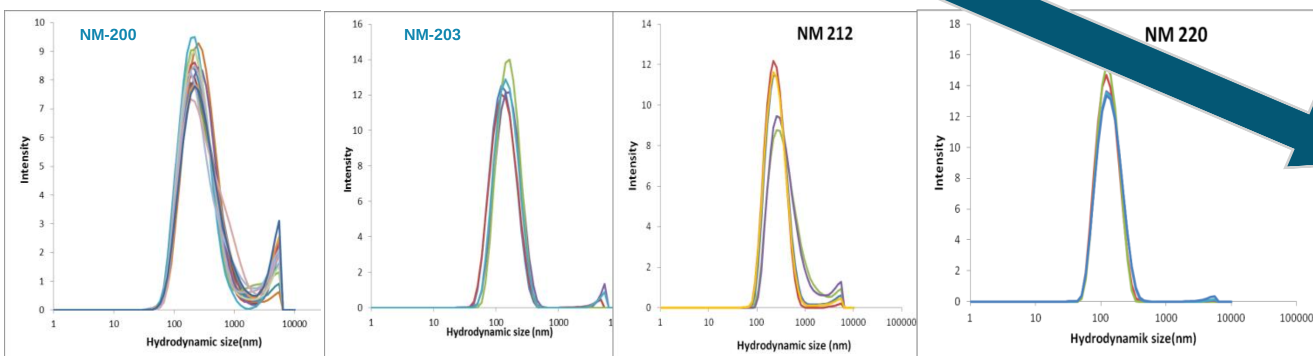

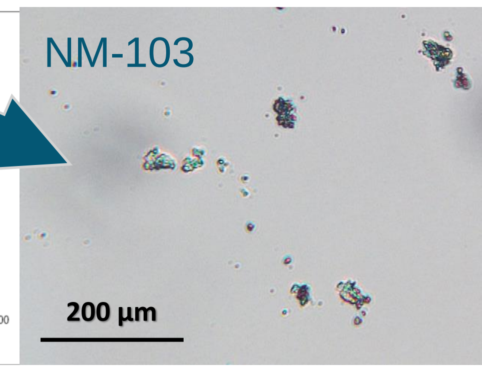

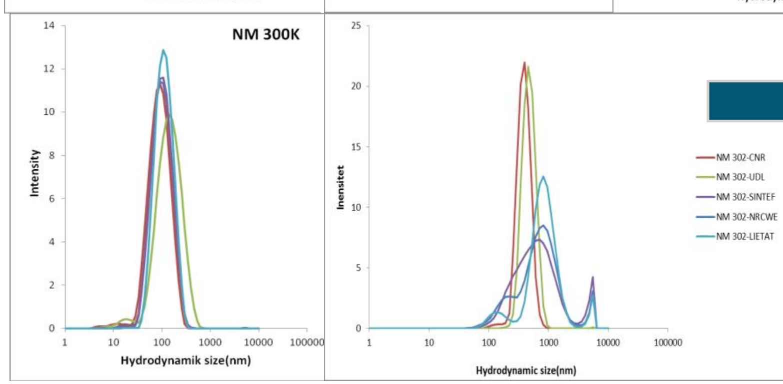

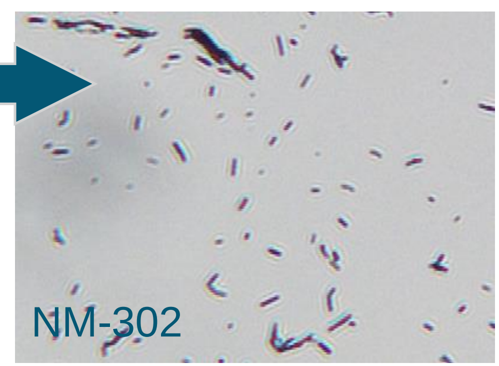

Performance on CNT and nanocellulose

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